of a naturally isolatable protein or fragment thereof, said *N*-terminal cysteine not being found in the naturally isolatable protein;

cl
conclude the intermediate conformation of said protein resulting directly from said ligation process.

cs 29. (once amended) The synthetically produced protein of claim 26, wherein said naturally isolatable protein is a mammalian protein.

REMARKS

Applicant thanks the Examiner for correcting the ambiguity of Applicant's amendment filed July 1, 2002 respecting the amendment to page 16 of the specification.

Applicant again thanks the Examiner for pointing out Applicant's error with respect to the priority claim. A corrective amendment has been made to the priority claim.

Applicant again thanks the Examiner for pointing out Applicant's failure to delete the Schemes and to correct the typographical error on page 9. Corrective amendments have been made.

Claim 11 has been broadened by amendment so as to include processes employing any variant residue, rather than just a cysteine residue. The amendment was made because support in the specification is express for the broader language.

Claim 24 has been narrowed by amendment so as to include the limitation that the protein has an intermediate conformation resulting directly from the ligation process. Support for this limitation is inherent in the specification.

Claim 29 has been amended so as to correct an error of dependency.

Rejection under 35 USC 112, first paragraph:

Claims 11-14, 24, 26, and 29-30 are rejected as unsupported by the specification because the specification allegedly does not teach that ligation process may be employed to produce a naturally isolatable protein that contains one or more **cysteine** residues that are not found in said naturally isolatable protein. Applicant traverses this rejection. In order to more clearly reflect the support of the specification, Claim 11 has been broadened by amendment so as to include the use of the ligation process for producing a isolatable protein that contains one or more **variant** residues that are not found in said naturally isolatable protein. This amended process would include the use of cysteine variants. Support for this teaching is found in the specification as follows:

> The first step is the chemoselective reaction of an unprotected synthetic peptide-α-thioester with another unprotected peptide segment containing an N-terminal Cys residue, to give a thioester-linked intermediate as the initial covalent product. Without change in the reaction conditions, this intermediate undergoes spontaneous, rapid intramolecular reaction to form a native peptide bond at the ligation site. The target full length polypeptide product is obtained in the desired final form without further manipulation. **The general synthetic access provided by the method of native chemical ligation greatly expands**

> **th scope of variation of the covalent structure of the protein molecule**. (Specification, page 7, first paragraph)

Further support for this teaching is found in the specification as follows:

> Straightforward total chemical synthesis of proteins represents the realization of an important objective of organic chemistry. It raises the exciting prospect of **unrestricted variation of protein covalent structure** made possible by general synthetic access, and will give new impetus to exploration of the structural basis of properties such as folding, stability, catalytic activity, binding, and biological action. (Specification, page 21, second paragraph)

Although an exemplary process employing a variant amino acid is not specifically disclosed in the specification of the present application, the concept is disclosed for employing such variant amino acids in the context of the ligation process. To a first approximation, the difficulty of the ligation is unaltered by the use of such variants. A chemist of ordinary skill would understand the specification of the present application to disclose this concept and would have no difficulty implementing the process, i.e., the process of substituting a variant amino acid residue into a naturally occurring protein using the disclosed ligation process. Applicant requests that the Examiner withdraw this basis of rejection.

Rejection under 35 USC 112, second paragraph:

Claims 29-31 have been rejected under Rejection under 35 USC 112, second paragraph for lack of clarity. More particularly, it is pointed out that these claims depend upon cancelled claims. Applicant's amendments obviate this basis for rejection.

Rejection under 35 USC 102(b):

Claims 8-10, 12-14, have been rejected as anticipated by WO Patent Application 96/34878 and by Dawson et al. Applicant's amendment correcting the priority claim obviates these bases for rejection.

Rejection under 35 USC 102(b):

Claims 24, 26, and 29-31 have been rejected as anticipated by Yamagishi. Applicant traverses this basis for rejection. Yamagishi discloses a recombinant protein having a variant amino acid. Claim 24 has been amended so as to include the limitation that the claimed protein has "an intermediate conformation resulting directly from the ligation process." Support for the intermediate conformation is inherent in the specification. In view of the fact that recombinant proteins, as disclosed by Yamagishi, are produced enzymatically, such recombinant proteins lack an intermediate conformation of the type that results from the ligation process disclosed in the present application. Accordingly, Claim 24, as amended, and claims 26 and 29-31 are novel over Yamagishi.

Summary:

Claims 8, 10-14, 24, 26 and 29-31 are pending. Claims 11, 24, and 29 have been amended. Claims 8, 10-14, 24, 26 and 29-31 are clear, fully supported by the specification, and unanticipated by the cited prior art. Allowance of Claims 8, 10-14, 24, 26 and 29-31 is respectfully requested.

Respectfully submitted,

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Date

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APPENDIX

VERSION OF SPECIFICATION AND CLAIMS WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Page 1, prior to "Field of Invention" on line 1, please delete the paragraph labeled "Cross-Reference to Related Application" and substitute the following new paragraph:

Cross-Reference to Related Application:

The present application is a continuation application of and claims priority, under 35 U.S.C. § 120 , from US patent application Serial No. [08/710,653] 08/945,997, filed February 12, 1998, which issued on February 6, 2001 as US Patent No. 6,184,344, and which was a national stage application under 35 U.S.C. § 371 of International Application No. PCT/US95/05668, filed May 4, 1995, which International Application was published in English.

Page 9, third paragraph, please make the following amendment:

Another aspect of the invention is directed to a method for producing an oligopeptide having a C-terminal thioester. The method admixes a resin having a linker with an unoxidized thiol with a Boc-amino acid succinimide ester under reaction conditions to produce a Boc-amino thioester-resin. An oligopeptide is then assembled onto the Boc-amino thioester-resin by stepwise solid phase peptide synthesis. When the oligopeptide is complete, the [the] Boc-amino thioester-resin is cleaved with HS to produce an oligopeptide having a C-terminal thiol. The C-terminal thiol is then converted to an oligopeptide having a C-terminal thioester.

APPENDIX

VERSION OF SPECIFICATION AND CLAIMS WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Amendments to claims 11, 24, and 29 as indicated as follows:

11. (once amended) The method of claim 8, wherein said desired protein is a derivative of a naturally isolatable protein that contains one or more [cysteine] variant residues that are not found in said naturally isolatable protein.

24. (twice amended) A synthetically produced protein of greater than about 35 amino acid residues, said protein having an intermediate conformation, wherein all of the residues of said protein are linked to adjacent residues via an amide bond, said protein being produced by the process of ligating together at least two oligopeptide fragments wherein:

(1) said first oligopeptide fragment having a length of 30 or more amino acid residues with a C-terminal non-β-branched amino acid residue modified as a C-terminal thioester; and

(2) said second oligopeptide fragment has an *N*-terminal cysteine having an unoxidized sulfhydryl side chain and a free amino group that is capable of forming a β-aminothioester linkage with said C-terminal thioester that rearranges to form an amide bond therein between; wherein said ligation results in the formation of an amide bond linking said first and second fragments, wherein said synthetically produced protein being a derivative of a naturally isolatable protein or fragment thereof, said *N*-terminal cysteine not being found in the naturally isolatable protein;

APPENDIX

VERSION OF SPECIFICATION AND CLAIMS WITH MARKINGS TO SHOW CHANGES MADE

the intermediate conformation of said protein resulting directly from said ligation process.

29. (once amended) The synthetically produced protein of Claim 26 [any of claims 27 or 28], wherein said naturally isolatable protein is a mammalian protein.